

Reanalysis of the effect of vitamin C on mortality in the CITRIS-ALI trial

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This supplement describes the extraction of mortality data from Figure 3 of the CITRIS-ALI trial report and the calculations for Cox regression.

The CITRIS-ALI trial report was published in:

Fowler AA, Truwit JD, Hite RD, Morris PE, DeWilde C, Priday A, et al.
Effect of vitamin C infusion on organ failure and biomarkers of inflammation and vascular injury in patients with sepsis and severe acute respiratory failure: the CITRIS-ALI randomized clinical trial. JAMA 2019;322:1261–1270.
<https://doi.org/10.1001/jama.2019.11825>

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Deaths in the CITRIS-ALI trial were shown as steps in Figure 3 in the CITRIS-ALI report. The figure is copied below to illustrate the measurement of deaths over the follow-up period.

When the number of participants is quite low, it is possible to back-calculate from the survival curve the number of participants (deaths) on each step. The size of the steps was measured from the digital figure as pixels and the scale of the figure as pixels was used to determine the number of deaths on each step. A spreadsheet was used to transform the pixel-values to the mortality scale on the Y-axis from 0% to 60%. The extraction of step sizes and the calculations on the spreadsheet are shown on page 3 (Table 1).

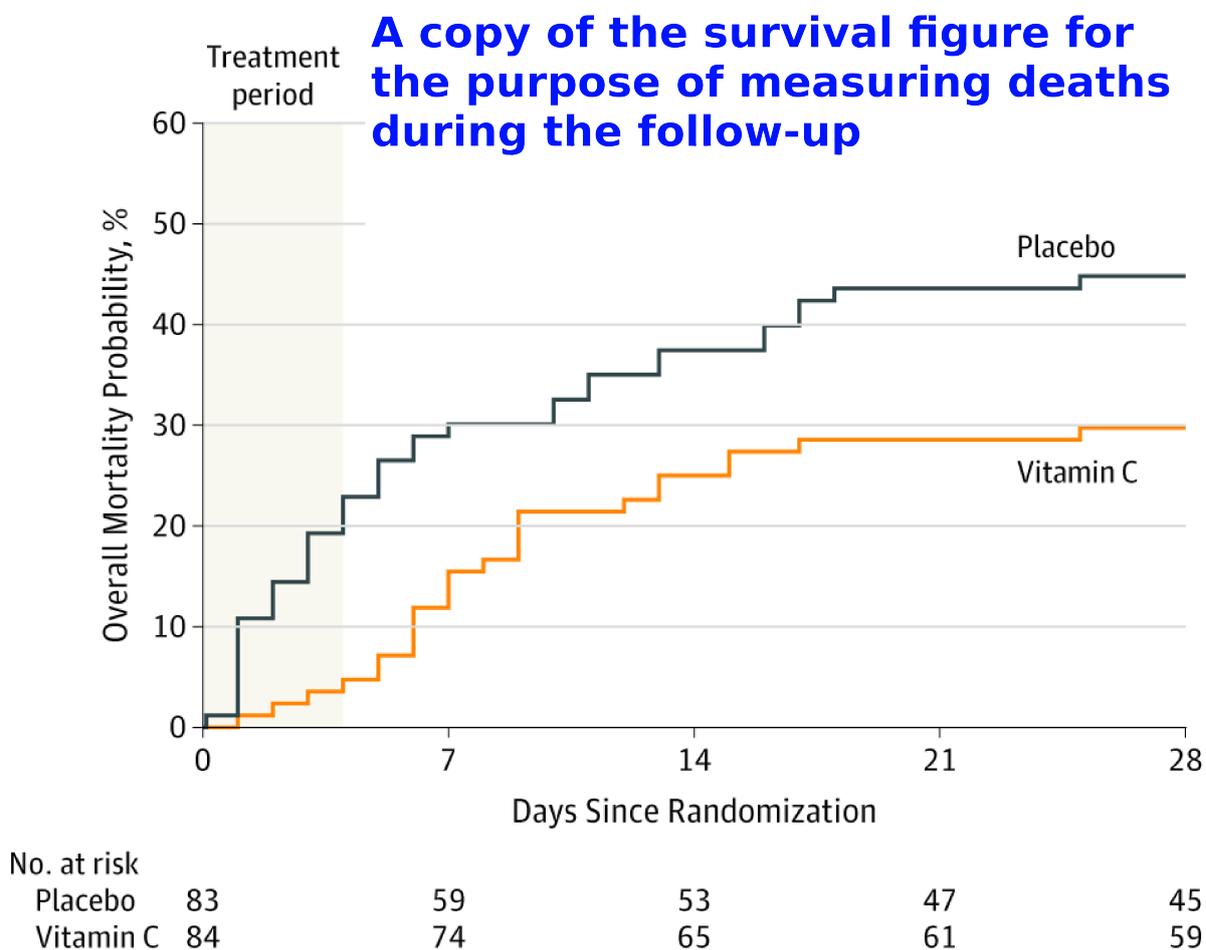


Table 1: All-cause mortality from randomization (Day 0) to Day 28 among patients with sepsis associated ARDS (from Fowler et al. Figure 3)

The left-hand side of this table shows the measurement of the steps in Figure 3.

The right-hand side shows the number of deaths by the end of the given day.

The number of patients (deaths) on each step could be inferred with great accuracy.

Scale		pixels		N:		Number of patients on each step Calculated		N cumulative Calculated		Death	
% mortality				Vitamin C	Placebo	Vitamin C	Placebo	Vitamin C	Placebo	vitC	Placebo
60		462		84	83						
0		2664									
Day	Vitamin C	Placebo		Vitamin C	Placebo	vitC	Placebo	Death			
0	2664	2664		0.00	0.00	0.00	0.00	1			
1	2619	2266		1.03	9.00	1.03	9.00	1			
2	2577	2134		1.99	11.99	0.96	2.99	1			
3	2534	1957		2.98	15.99	0.98	4.00	1			
4	2490	1824		3.98	19.00	1.01	3.01	1			
5	2401	1692		6.02	21.98	2.04	2.99	1			
6	2227	1604		10.00	23.97	3.98	1.99	1			
7	2096	1559		13.00	24.99	3.00	1.02	1			
8	2053	1559		13.98	24.99	0.98	0.00	1			
9	1877	1559		18.01	24.99	4.03	0.00	1			
10	1877	1470		18.01	27.00	0.00	2.01	1			
11	1877	1379		18.01	29.06	0.00	2.06	1			
12	1835	1379		18.97	29.06	0.96	0.00	1			
13	1746	1290		21.01	31.07	2.04	2.01	1			
14	1746	1290		21.01	31.07	0.00	0.00	1			
15	1660	1290		22.98	31.07	1.97	0.00	1			
16	1660	1199		22.98	33.13	0.00	2.06	1			
17	1616	1109		23.99	35.17	1.01	2.04	1			
18	1616	1065		23.99	36.16	0.00	1.00	1			
19	1616	1065		23.99	36.16	0.00	0.00	1			
20	1616	1065		23.99	36.16	0.00	0.00	1			
21	1616	1065		23.99	36.16	0.00	0.00	1			
22	1616	1065		23.99	36.16	0.00	0.00	1			
23	1616	1065		23.99	36.16	0.00	0.00	1			
24	1616	1065		23.99	36.16	0.00	0.00	1			
25	1573	1020		24.97	37.18	0.98	1.02	1			
26	1573	1020		24.97	37.18	0.00	0.00	1			
27	1573	1020		24.97	37.18	0.00	0.00	1			
28	1573	1020		24.97	37.18	0.00	0.00	1			
Alive at the end of day 28:						59.03	45.82	0			
						Total deaths:	25.0	37.2			

Cox regression: two time periods of vitamin C effects

Printouts of the calculation of the Cox regression models.

The **base** model indicates model with only *one uniform vitamin C effect* over the 28-day follow-up.

```
> CITRIX_S <- Surv(CITRIS$Day, CITRIS$Censored)
> CITRIX_S
[1] 1 1 1 1 1 1 1 1 1 2 2 2 3 3 3 3 4 4 4 5 5 5 6 6 7 10
[27] 10 11 11 13 13 16 16 17 17 18 25 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+
[53] 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+
[79] 28+ 28+ 28+ 28+ 28+ 1 2 3 4 5 5 6 6 6 6 7 7 7 8 9 9 9 9 12 13 13
[105] 15 15 17 25 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+
[131] 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+
[157] 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+
>
>
> base <- coxph(CITRIX_S ~ CITRIS$VitC, method = "efron")
> summary(base)
Call:
coxph(formula = CITRIX_S ~ CITRIS$VitC, method = "efron")

n= 167, number of events= 62

              coef exp(coef) se(coef)      z Pr(>|z|)
CITRIS$VitC -0.5639   0.5690  0.2590 -2.177  0.0295 *
---

              exp(coef) exp(-coef) lower .95 upper .95
CITRIS$VitC   0.569      1.758   0.3425  0.9453

Concordance= 0.579 (se = 0.031 )
Likelihood ratio test= 4.85 on 1 df,  p=0.03
Wald test               = 4.74 on 1 df,  p=0.03
Score (logrank) test = 4.87 on 1 df,  p=0.03

> lrtest(base)
Likelihood ratio test

Model 1: CITRIX_S ~ CITRIS$VitC
Model 2: CITRIX_S ~ 1
#Df LogLik Df Chisq Pr(>Chisq)
1 1 -301.84
2 0 -304.27 -1 4.8528 0.0276 *
---
```

Adding a second vitamin C effect time period. The first lines of the code divide the follow-up between days 1 and 2, ie. the first period is from the beginning to the end of day 1 and second is from day 2 to day 28. The next lines divide the follow-up between days 2 and 3, and so on.

```
> days1 <- survSplit(Surv(CITRIS$Day, CITRIS$Censored) ~ ., cut=c(1.5),
episode= "tgroup", data =CITRIS)
> days1
> days1b <- coxph(Surv(tstart, tstop, event) ~ VitC:strata(tgroup), data=days1,
method = "efron")
> days1b
Call:
coxph(formula = Surv(tstart, tstop, event) ~ VitC:strata(tgroup),
      data = days1, method = "efron")
```

	coef	exp(coef)	se(coef)	z	p
VitC:strata(tgroup)tgroup=1	-2.2547	0.1049	1.0541	-2.139	0.0324
VitC:strata(tgroup)tgroup=2	-0.3477	0.7063	0.2782	-1.250	0.2114

Likelihood ratio test=9.4 on 2 df, p=0.009113

n= 324, number of events= 62

```
> lrtest(base,days1b)
```

Likelihood ratio test

```
Model 1: CITRIX_S ~ CITRIS$VitC
Model 2: Surv(tstart, tstop, event) ~ VitC:strata(tgroup)
#Df LogLik Df Chisq Pr(>Chisq)
1 1 -301.84
2 2 -299.57 1 4.5432 0.033
---
```

```
> days2 <- survSplit(Surv(CITRIS$Day, CITRIS$Censored) ~ ., , cut=c(2.5),
episode= "tgroup", data =CITRIS)
> days2b <- coxph(Surv(tstart, tstop, event) ~ VitC:strata(tgroup), data=days2,
method = "efron")
> days2b
Call:
coxph(formula = Surv(tstart, tstop, event) ~ VitC:strata(tgroup),
      data = days2, method = "efron")
```

	coef	exp(coef)	se(coef)	z	p
VitC:strata(tgroup)tgroup=1	-1.8690	0.1543	0.7638	-2.447	0.0144
VitC:strata(tgroup)tgroup=2	-0.2826	0.7539	0.2890	-0.978	0.3282

Likelihood ratio test=9.67 on 2 df, p=0.007938

n= 320, number of events= 62

```
> lrtest(base,days2b)
```

Likelihood ratio test

```
Model 1: CITRIX_S ~ CITRIS$VitC
Model 2: Surv(tstart, tstop, event) ~ VitC:strata(tgroup)
#Df LogLik Df Chisq Pr(>Chisq)
1 1 -301.84
2 2 -299.44 1 4.8194 0.028
```

```
> days3 <- survSplit(Surv(CITRIS$Day, CITRIS$Censored) ~ ., cut=c(3.5),
episode= "tgroup", data =CITRIS)
> days3b <- coxph(Surv(tstart, tstop, event) ~ VitC:strata(tgroup), data=days3,
method = "efron")
```

```
> days3b
Call:
coxph(formula = Surv(tstart, tstop, event) ~ VitC:strata(tgroup),
      data = days3, method = "efron")
```

	coef	exp(coef)	se(coef)	z	p
VitC:strata(tgroup)tgroup=1	-1.7741	0.1696	0.6293	-2.819	0.00481
VitC:strata(tgroup)tgroup=2	-0.1569	0.8548	0.3051	-0.514	0.60700

```
Likelihood ratio test=11.37 on 2 df, p=0.003394
n= 315, number of events= 62
```

```
> lrtest(base,days3b)
Likelihood ratio test
```

```
Model 1: CITRIX_S ~ CITRIS$VitC
Model 2: Surv(tstart, tstop, event) ~ VitC:strata(tgroup)
```

	#Df	LogLik	Df	Chisq	Pr(>Chisq)
1	1	-301.84			
2	2	-298.58	1	6.5185	0.0106

```
---
```

```
> days4 <- survSplit(Surv(CITRIS$Day, CITRIS$Censored) ~ ., cut=c(4.5),
episode= "tgroup", data =CITRIS)
> days4b <- coxph(Surv(tstart, tstop, event) ~ VitC:strata(tgroup), data=days4,
method = "efron")
```

```
> days4b
Call:
coxph(formula = Surv(tstart, tstop, event) ~ VitC:strata(tgroup),
      data = days4, method = "efron")
```

	coef	exp(coef)	se(coef)	z	p
VitC:strata(tgroup)tgroup=1	-1.67644	0.18704	0.55027	-3.047	0.00231
VitC:strata(tgroup)tgroup=2	-0.04942	0.95178	0.32125	-0.154	0.87775

```
Likelihood ratio test=12.5 on 2 df, p=0.00193
```

```
n= 311, number of events= 62
```

```
> lrtest(base,days4b)
Likelihood ratio test
```

```
Model 1: CITRIX_S ~ CITRIS$VitC
Model 2: Surv(tstart, tstop, event) ~ VitC:strata(tgroup)
```

	#Df	LogLik	Df	Chisq	Pr(>Chisq)
1	1	-301.84			
2	2	-298.02	1	7.6459	0.0056

```
---
```

```
> summary(days4b)
```

```
Call:
coxph(formula = Surv(tstart, tstop, event) ~ VitC:strata(tgroup),
      data = days4, method = "efron")
```

	exp(coef)	exp(-coef)	lower .95	upper .95
VitC:strata(tgroup)tgroup=1	0.1870	5.346	0.06361	0.5499
VitC:strata(tgroup)tgroup=2	0.9518	1.051	0.50710	1.7864

```

> days5 <- survsplit(Surv(CITRIS$Day, CITRIS$Censored) ~ ., cut=c(5.5),
episode= "tgroup", data =CITRIS)
> days5b <- coxph(Surv(tstart, tstop, event) ~ VitC:strata(tgroup), data=days5,
method = "efron")
> days5b
Call:
coxph(formula = Surv(tstart, tstop, event) ~ VitC:strata(tgroup),
      data = days5, method = "efron")

```

	coef	exp(coef)	se(coef)	z	p
VitC:strata(tgroup)tgroup=1	-1.43833	0.23732	0.46076	-3.122	0.0018
VitC:strata(tgroup)tgroup=2	0.03703	1.03773	0.34544	0.107	0.9146

Likelihood ratio test=12.08 on 2 df, p=0.002385
n= 306, number of events= 62

```
> lrtest(days5b)
```

Likelihood ratio test

Model 1: Surv(tstart, tstop, event) ~ VitC:strata(tgroup)

Model 2: Surv(tstart, tstop, event) ~ 1

#Df	LogLik	Df	Chisq	Pr(>Chisq)
1	-298.23			
2	-304.27	-2	12.077	0.002385 **

```
> lrtest(base,days5b)
```

Likelihood ratio test

Model 1: CITRIX_S ~ CITRIS\$VitC

Model 2: Surv(tstart, tstop, event) ~ VitC:strata(tgroup)

#Df	LogLik	Df	Chisq	Pr(>Chisq)
1	-301.84			
2	-298.23	1	7.2242	0.0071

```

> days6 <- survsplit(Surv(CITRIS$Day, CITRIS$Censored) ~ ., cut=c(6.5),
episode= "tgroup", data =CITRIS)
> days6b <- coxph(Surv(tstart, tstop, event) ~ VitC:strata(tgroup), data=days6,
method = "efron")
> days6b
Call:
coxph(formula = Surv(tstart, tstop, event) ~ VitC:strata(tgroup),
      data = days6, method = "efron")

```

```

> days6b <- coxph(Surv(tstart, tstop, event) ~ VitC:strata(tgroup), data=days6,
method = "efron")
> days6b
Call:
coxph(formula = Surv(tstart, tstop, event) ~ VitC:strata(tgroup),
      data = days6, method = "efron")

```

```

Call:
coxph(formula = Surv(tstart, tstop, event) ~ VitC:strata(tgroup),
      data = days6, method = "efron")

```

	coef	exp(coef)	se(coef)	z	p
VitC:strata(tgroup)tgroup=1	-1.03195	0.35631	0.37660	-2.740	0.00614
VitC:strata(tgroup)tgroup=2	-0.04803	0.95310	0.37897	-0.127	0.89914

Likelihood ratio test=8.34 on 2 df, p=0.01543
n= 300, number of events= 62

```
> lrtest(base,days6b)
```

Likelihood ratio test

Model 1: CITRIX_S ~ CITRIS\$VitC

Model 2: Surv(tstart, tstop, event) ~ VitC:strata(tgroup)

#Df	LogLik	Df	Chisq	Pr(>Chisq)
1	-301.84			
2	-300.10	1	3.4903	0.061

```
> days7 <- survsplit(Surv(CITRIS$Day, CITRIS$Censored) ~ ., cut=c(7.5),
episode= "tgroup", data =CITRIS)
> days7b <- coxph(Surv(tstart, tstop, event) ~ VitC:strata(tgroup), data=days7,
method = "efron")
> days7b
```

Call:

```
coxph(formula = Surv(tstart, tstop, event) ~ VitC:strata(tgroup),
      data = days7, method = "efron")
```

	coef	exp(coef)	se(coef)	z	p
VitC:strata(tgroup)tgroup=1	-0.8167	0.4419	0.3421	-2.387	0.017
VitC:strata(tgroup)tgroup=2	-0.1868	0.8296	0.4083	-0.457	0.647

Likelihood ratio test=6.26 on 2 df, p=0.04371

n= 296, number of events= 62

```
> lrtest(base,days7b)
```

Likelihood ratio test

Model 1: CITRIX_S ~ CITRIS\$VitC

Model 2: Surv(tstart, tstop, event) ~ VitC:strata(tgroup)

	#Df	LogLik	Df	Chisq	Pr(>Chisq)
--	-----	--------	----	-------	------------

1	1	-301.84			
---	---	---------	--	--	--

2	2	-301.14	1	1.4073	0.23
---	---	---------	---	--------	------

Calculation of the NNT for the 4-day duration of vitamin C supplementation

```
> prop.test(c(4,19),c(84,83),correct=FALSE)
```

2-sample test for equality of proportions without continuity correction

```
data: c(4, 19) out of c(84, 83)
X-squared = 12, df = 1, p-value = 7e-04
alternative hypothesis: two.sided
95 percent confidence interval:
-0.28251 -0.08009
sample estimates:
 prop 1  prop 2
0.04762 0.22892
```

```
>
> (Difference = 0.22892 - 0.04762 )
[1] 0.1813
> (NNT = 1/Difference)
[1] 5.516
>
> # 95% CI for NNT
>
> (1/0.28251)
[1] 3.54
> (1/0.08009)
[1] 12.49
```

**Cox regression over first four days:
the effect of vitamin C during the actual vitamin C administration.**

```
CITRIX4Day_S <- Surv(CITRIS4Day$Day, CITRIS4Day$Censored)
> CITRIX4Day_S
[1] 1 1 1 1 1 1 1 1 1 2 2 2 3 3 3 3 4 4 4 5+ 5+ 5+ 6+ 6+ 7+ 10+
[27] 10+ 11+ 11+ 13+ 13+ 16+ 16+ 17+ 17+ 18+ 25+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+
[53] 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+
[79] 28+ 28+ 28+ 28+ 28+ 1 2 3 4 5+ 5+ 6+ 6+ 6+ 6+ 7+ 7+ 7+ 8+ 9+ 9+ 9+ 9+ 12+ 13+ 13+
[105] 15+ 15+ 17+ 25+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+
[131] 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+
[157] 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+ 28+
>
```

```
>
> Only4D <- coxph(CITRIX4Day_S ~ CITRIS4Day$VitC, method = "efron")
> summary(Only4D)
Call:
coxph(formula = CITRIX4Day_S ~ CITRIS4Day$VitC, method = "efron")
```

n= 167, number of events= 23

	coef	exp(coef)	se(coef)	z	Pr(> z)
CITRIS4Day\$VitC	-1.6764	0.1870	0.5503	-3.047	0.00231 **

	exp(coef)	exp(-coef)	lower .95	upper .95
CITRIS4Day\$VitC	0.187	5.346	0.06361	0.5499

Concordance= 0.683 (se = 0.042)
Likelihood ratio test= 12.48 on 1 df, p=4e-04
Wald test = 9.28 on 1 df, p=0.002
Score (logrank) test = 11.66 on 1 df, p=6e-04

```
> lrtest(Only4D)
Likelihood ratio test
```

```
Model 1: CITRIX4Day_S ~ CITRIS4Day$VitC
Model 2: CITRIX4Day_S ~ 1
#Df LogLik Df Chisq Pr(>Chisq)
1 1 -109.89
2 0 -116.13 -1 12.475 0.00041
```

Risk ratio and Fisher exact test over the first four days

Over the first four days when vitamin C was administered, there were 4 deaths in the vitamin C group of 84 participants and 19 deaths in the placebo group of 83 participants.

This leads to a risk ratio of $RR = 0.21$ (95% CI 0.07-0.59):

```
> riskratio(4, 19, 84, 83, conf.level=0.95, p.calc.by.independence=TRUE)
```

	Disease	Nondisease	Total
Exposed	4	80	84
Nonexposed	19	64	83

Risk ratio estimate and its significance probability

```
data: 4 19 84 83
```

```
p-value = 0.00070
```

```
95 percent confidence interval:
```

```
0.0739 0.585
```

```
sample estimates:
```

```
[1] 0.208
```

```
> CITRIS4D <- matrix(c(4, 19, 80, 64),nrow=2)
```

```
> CITRIS4D
```

```
      [,1] [,2]  
[1,]    4  80  
[2,]   19  64
```

```
> fisher.test(CITRIS4D)
```

Fisher's Exact Test for Count Data

```
data: CITRIS4D
```

```
p-value = 0.00065
```

```
alternative hypothesis: true odds ratio is not equal to 1
```

```
95 percent confidence interval:
```

```
0.040 0.546
```

```
sample estimates:
```

```
odds ratio
```

```
0.17
```